

Geographic Distribution and Genetic Variation of a Vulnerable Conifer Species, *Picea koyamae* (Pinaceae)

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Picea koyamae is a species which is distributed scarcely and is threatened to extinction, though the details are not well known. We investigated the geographical distribution and genetic variation of this species. Seven habitats over the Yatsugatake mountains and Akaishi mountains were confirmed in this study, and it was estimated that in these areas combined there were less than 1,000 living mature trees. Genetic diversity within and among 4 populations was analyzed using allozymes. The mean genetic variation within populations was in the range of general long-lived tree species. However, genetic variation in the Karamatsu-sawa population at Yatsugatake mountains showed a rather low value. It is conceivable that this population has experienced a bottleneck effect. The genetic differentiation among 4 populations was found to be at an intermediate level between continuously distributing conifers and discontinuously distributing ones. In this study, "*P. shirasawae*" was suggested to be merely a morphological variation within a population of *P. koyamae*, and was treated as *P. koyamae* in our results.

Key words: Akaishi mountains, allozyme, genetic diversity, habitat, *Picea koyamae*, *Picea shirasawae*, Yatsugatake mountains

Picea koyamae Shiras. (Yatsugatake-tôhi in Japanese) is a tall conifer tree species which is distributed in Honshu Island, Japan. In 1911 it was discovered in the Yatsugatake mountains area (Shirasawa & Koyama 1913). Since then, *P. koyamae* has been believed to be distributed only on Yatsugatake mountains (Farjon 1990, Yamazaki 1995). Currently, there are less than 500 living mature trees in the Yatsugatake mountains area, and is listed as "threatened with extinction" in a

report on Red Data Plants (Farjon & Page 1999, Environmental Agency of Japan 2000).

The genus *Picea* is one of the most important tree groups in Japanese flora to come out of the Last Glacial Age, and fossils classified into *P. koyamae* or "*P. shirasawae* Hayashi" have been discovered in very wide area on Honshu Island (Minaki 1987). In addition, more than 10 genus *Picea* species, including now-extinct ones, have been reported based on fossil records in Honshu (Suzuki

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TABLE 1. Classification of Sect. *Picea* around the Japan Sea, in Farjon (1990), Shimizu(1992) and Yamazaki (1995).

Farjon (1990)	Shimizu(1992)	Yamazaki (1995)	Type locality
<i>P. alcoquitana</i> (Veitch ex Lindl.) Carrière “ <i>alcockiana</i> ” §	<i>P. alcoquitana</i> (Veitch ex Lindl.) Carrière “ <i>alcockiana</i> ” §	<i>P. alcoquitana</i> (Veitch ex Lindl.) Carrière “ <i>alcockiana</i> ” §	Yamanashi, Japan
<i>P. alcoquitana</i> var. <i>acicularis</i> (Shiras. & Koyama) Fitschen	<i>P. koyamae</i> var. <i>acicularis</i> (Shiras. & Koyama) T. Shimizu	<i>P. shirasawae</i> Hayashi	Nagano, Japan
<i>P. alcoquitana</i> var. <i>reflexa</i> (Shiras. & Koyama) Fitschen	<i>P. alcoquitana</i> var. <i>reflexa</i> (Shiras. & Koyama) Fitschen	<i>P. alcoquitana</i> var. <i>reflexa</i> (Shiras. & Koyama) Fitschen	Nagano, Japan
<i>P. glehnii</i> (F. Schmidt) Mast.	<i>P. glehnii</i> (F. Schmidt) Mast.	<i>P. glehnii</i> (F. Schmidt) Mast.	Sachalin, Russia
<i>P. koraiensis</i> Nakai	No description	No description	Hamkyongpuk-do, North Korea
<i>P. koraiensis</i> var. <i>pungshanensis</i> (Uyeki ex Nakai) Schmidt-Vogt ex Farjon	No description	No description	Ryangkang-do, North Korea
<i>P. koyamai</i> Shiras.	<i>P. koyamae</i> Shiras. “ <i>koyamai</i> ”	<i>P. koyamae</i> Shiras. “ <i>koyamai</i> ”	Nagano, Japan
<i>P. maximowiczii</i> Regel ex Mast.	<i>P. maximowiczii</i> Regel	<i>P. maximowiczii</i> Regel ex Carrière	Yamanashi §§, Japan
<i>P. maximowiczii</i> var. <i>senanensis</i> Hayashi	<i>P. maximowiczii</i> var. <i>senanensis</i> Hayashi	Described as an individual variation	Nagano, Japan
<i>P. obovata</i> Ledeb.	No description	No description	Altay, Russia
<i>P. torano</i> (K. Koch) Koehne \$\$\$	<i>P. torano</i> (K. Koch) Koehne \$\$\$	<i>P. polita</i> (Sieb. & Zucc.) Carrière \$\$\$	Tokyo \$\$\$\$, Japan

§ Often called as *P. bicolor* Maxim. in Japan.

§§ Considered to be an error in Nagano.

\$\$\$ Also named for a specimen collected by Siebold.

\$\$\$\$ Collected from cultivated tree.

1991, LePage 2001), though the details of their phylogenetic relationships are unknown.

There are several problems with the classification of *Picea koyamae* and its sibling species. Around the Japan Sea, six or seven species classified into Sect. *Picea* have been reported by Farjon (1990), Shimizu (1992) and Yamazaki (1995) (Table 1, Fig. 1). Some botanists have pointed out that *P. koyamae*, *P. koraiensis*, and *P. obovata* are very similar (Wilson 1916, Wright 1955). In particular, there was a case that *P. koraiensis* was included with *P. koyamae* as one species (Fu *et al.* 1978), though no detailed examination had been made.

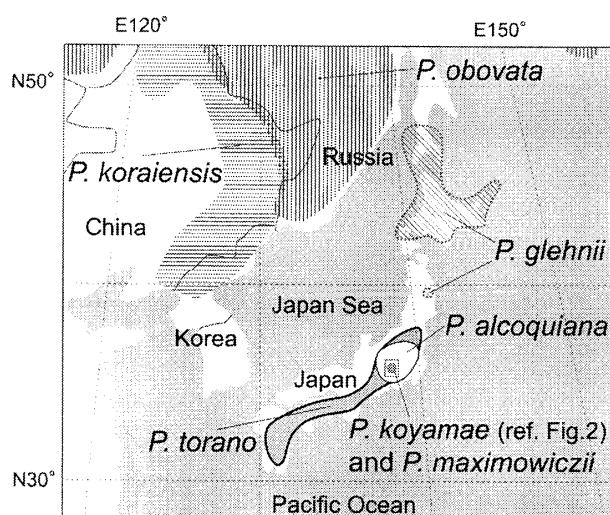


FIG. 1. Distribution of Sect. *Picea* species around the Japan Sea. The species names are followed by description of Farjon (1990).

On the other hand, "*Picea shirasawae*" (Hime-matsuhada in Japanese) has been sometimes treated as an independent species (Hayashi 1960b, Yamazaki 1995). However, many botanists have classified it as a variety of *P. koyamae* (Shimizu 1989) or *P. alcoquiana* (Farjon 1990). "*Picea shirasawae*" was discovered in the same geographical area in which *P. koyamae* was discovered, and the discoveries of both were published in the same report (Shirasawa & Koyama 1913). "*Picea shirasawae*" resembles *P. koyamae*, and has been reported to be morphologi-

cally distinguished from *P. koyamae* by longer cone length (8 - 12 cm), longer leaf length (13 - 18 mm) and branchlets color (orange-brown in pubescence) (Hayashi 1960a). However, Shimizu (1992) pointed out that there was not a difference other than the form and size of cone between those two taxa. In addition, Kobayashi *et al.* (2000) reported a total of about 2500 bases of nucleotide sequences in four regions of chloroplast DNA for one *P. koyamae* individual and one "*P. shirasawae*" individual, and their sequences were identical with each other though they showed some difference from the sequences of other four Japanese Sect. *Picea* species. This might support the classification of Shimizu (1992).

In this study, we confirmed the present geographical distribution of *Picea koyamae* and analyzed their genetic variation using allozymes (enzyme polymorphisms) without distinguishing "*P. shirasawae*" as a separate species according to the classification by Shimizu (1989, 1992).

Materials and Methods

Plant materials

Based on descriptions in past reports (Hayashi 1960a, Yamazaki 1965, Katsuki & Seido 1999), we considered *Picea koyamae* to be geographically distributed in the Yatsugatake mountains and Akaishi mountains areas. Then, we confirmed their current habitats by means of field survey and direct examination to identify the species.

After this survey, we studied the genetic variation in allozymes of the four populations (Fukisawa and Karamatsu-sawa populations in Yatsugatake mountains; Ohdaira and Tenshi-iwa populations in Akaishi mountains) from which we had obtained a sufficient number of samples of *Picea koyamae* individuals (Fig. 2).

For allozyme analysis, we collected young branches of about 30 individuals from each of the four populations in June, 2000. Collected branches

were stored at -80°C until the enzymes were extracted.

Allozyme analysis

Procedures for enzyme extraction and polyacrylamide gel electrophoresis followed those described in Tsumura *et al.* (1990) and Tsumura & Ohba (1993), except that 100 mg of young leaf tissue from each tree and 80 mg of polyvinyl-polypyrrolidone were ground to a paste with a mortar and pestle in 1 ml of extraction buffer (100 mM Tris-HCl pH 7.5, 23.4% glycerol, 0.6% tween 80, 1.2 mM dithiothreitol, 0.6 mM NAD, 0.5 mM NADP, 0.5% 2-mercaptoethanol, and 0.08% albumin).

Eight enzyme systems were employed: diaphorase (*Dia*, EC 1.6.4.3.), fumarate hydratase (*Fm*, EC 4.2.1.2.), glycerol-2-dehydrogenase (*G2dh*, EC 1.1.1.29.), glutamate dehydrogenase (*Gdh*, EC 1.4.1.2.), glutamate oxaloacetate transaminase (*Got*, EC 2.6.1.1.), phosphoglucosmutase (*Pgm*, EC 2.7.5.1.), shikimate dehydrogenase (*Shdh*, EC 1.1.1.25.), and UDP glucose pyrophosphorylase (*Ugpp*, EC 2.7.7.9.). The staining method for *Ugpp* is described in Harris & Hopkinson (1976), and those for the other enzyme systems follow the methods set out by Tsumura *et al.* (1990) and Tsumura & Ohba (1993).

Statistical analysis

Levels of genetic variation were calculated for each of the populations according to a number of parameters including: the proportion of polymorphic loci (*P*) at the 95% criterion, the average number of alleles per locus (*Na*), the effective number of alleles per locus (*Ne*; Kimura & Crow 1964), the observed heterozygosity (*Ho*), and the expected unbiased heterozygosity under Hardy-Weinberg equilibrium (*He*; Nei 1978, Nei & Roychoudhury 1974).

F-statistics (*Fis*) (Wright 1951) that represent the deviation of genotypic proportions from the Hardy-Weinberg expectation were calculated for polymorphic loci in each population. Statistical analysis of deviations from such expectations was made using the χ^2 test (Li & Horvitz 1953).

Gene differentiation within and among populations were calculated according to the following genetic diversity statistics (Nei 1973, Nei & Chesser 1983): the total population gene diversity, *Ht*; the average gene diversities within populations, *Hs*; and the relative extent of gene differentiation among populations, *Gst*.

Nei's unbiased standard genetic distances were calculated for all population pairs (Nei 1972, Nei & Roychoudhury 1974), and clusters were analyzed using the unweighted pair-group method with arithmetic averages (UPGMA; Sneath & Sokal 1973).

The above calculations were carried out using

TABLE 2. The confirmed populations of *Picea. koyamae* in this study.

Mountains	Habitat	Location	Altitude (m)	Population size [§]
Yatsugatake	Fuki-sawa	Nishidake-305 ^{§§} , Fujimi, Nagano	1800	30
	Karamatsu-sawa	Nishidake-310 ^{§§} , Fujimi, Nagano	1650	100
Akaishi	Ohdaira	Nirasaki-66 ^{§§§} , Hakushu, Yamanashi	1600	10
	Kitazawa-tôge	Miwa-278 ^{§§} , Hase, Nagano	1500	<10
	Mikobuchi	Inasato-78 ^{§§} , Hase, Nagano	1450	<10
	Koma-sawa	Inasato-85 ^{§§} , Hase, Nagano	1450	<10
	Tenshi-iwa	Ohkawara-76 ^{§§} , Ohshika, Nagano	1750	50

[§] Roughly estimated number of mature trees (bigger than about 20cm DBH).

^{§§} Forest compartment assigned by the National Forest Agency.

^{§§§} Forest compartment assigned by Yamanashi Prefecture.

GENESTRUT (Constantine *et al.* 1994) and DISPAN (Ota 1993) computer software.

Results

Present geographical distribution

In our survey, a total of about 220 mature trees of *Picea koyamae* at 7 habitats were confirmed (Fig. 2, Table 2).

In the Yatsugatake mountains area, Fuki-sawa was thought to be the type locality of the *Picea koyamae* collected by Mr. Koyama in 1911 (Wilson 1916), but this forest suffered heavy damage by the Isewan typhoon in 1959. At present, over 1,000 young trees and about 30 mature trees (bigger than about 20cm DBH) with cones can be observed in Fuki-sawa. Karamatsu-sawa, where there are about 100 mature trees of *P. koyamae*, is the most well known habitat (Hayashi 1960a). The National Forest Agency registered it as a gene resource preservation forest in 1987. In this population, the diameter dis-

tribution of mature trees shows a peak at 30-40cm. The age of a big tree (DBH = 50.0cm; H = 22.3m) damaged in 2001 by typhoon was estimated to be 103 years.

In the Akaishi mountains area, Ohdaira is a locality that has been confirmed recently as a habitat of *Picea koyamae* and *P. maximowiczii* (Katsuki & Seido 1999), and about 30 individuals of *P. koyamae* with a height over 1.3m were observed in a small area (14ha). The number of mature trees seems to be about 10. Kitazawa-tôge, Mikobuchi, and Koma-sawa are the localities reported by Hayashi (1960a). However, the numbers of samples of *P. koyamae* in those habitats were insufficient for the present allozyme analysis, since the number of mature trees in each of those localities appeared to be less than 10. Tenshi-iwa was reported by Yamazaki (1965) to be the southern limit locality of "*P. shirasawae*". Though the details of this population were not reported in the previous paper, our field survey clarified that there are more than 50 mature trees of *P. koyamae* in this locality.

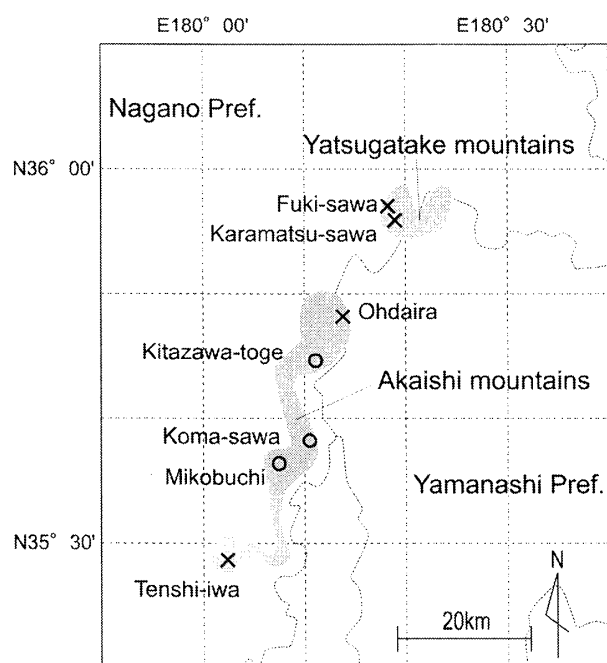


FIG. 2. Reported distribution and confirmed habitats of *Picea koyamae*. Hatched area, geographical distribution described in Hayashi 1960a, Katsuki & Seido 1999 and Yamazaki 1965. X mark, habitat in which samples were collected. Circle, observed habitat.

Genetic variation in 4 populations at two mountainous areas

The sampled individuals for allozyme analysis were mainly mature trees in Fuki-sawa, Karamatsu-sawa and Tenshi-iwa and the mixture of mature trees and young trees in Ohdaira. Ten putative loci were detected on eight enzyme systems: two loci each for *Dia* and *Got* and one locus for the others. At the 95% criterion, four loci (*Dia-2*, *G2d*, *Pgm*, and *Shdh*) were polymorphic; one locus (*Dia-1*) was classified as monomorphic with very little variation; and the other five loci (*Fm*, *Gdh*, *Got-1*, *Got-2*, and *Ugpp*) showed no genetic variation at all (Table 3). Table 4 shows a summary of the degree of genetic variation. It can be seen that three populations (Fuki-sawa, Ohdaira and Tenshi-iwa) showed approximately the same degree of genetic variation ($He=0.185-0.196$). The Karamatsu-sawa population appeared to hold slightly lower variation

($H_e=0.136$) than those three populations, though the difference was not statistically significant.

The estimated values of F_{IS} for polymorphic loci are summarized in Table 5. A significant F_{IS} value is seen only for the *Pgm* locus in the Ohdaira population; other F_{IS} values were not significant.

Table 6 shows the levels of genetic differentiation among the 4 populations. The average G_{ST} value over all loci was 0.067.

Genetic distances among the 4 populations were between 0.001 (Ohdaira vs. Tenshi-iwa) and 0.045 (Fuki-sawa vs. Karamatsu-sawa) (Table 7). Based on standard genetic distances, the dendrogram by the UPGMA method is shown in Fig. 3. The bootstrap probability suggested combining the Ohdaira and Tenshi-iwa populations as the most closely related at 69%.

Discussion

In this study, 7 habitats of *Picea koyamae* were confirmed (Table 2), but the number of trees in Akaishi mountains area were shown to be underestimated, since our survey was not conducted in all areas of Akaishi mountains. Furthermore, in some cases, a stand of *P. koyamae* individuals occurred on a steep slope, and we could not reach such a habitat. Note *et al.* (1999) reported many *P. koyamae* individuals standing on steep slopes or near cliffs on the other side of the Mibu River at Mikobuchi by viewing them with binoculars, but it was not possible to approach these trees and identify their species directly. Table 2 includes only the number of mature trees we could identify directly by touching them. Consequently, it is supposed that in the area of the Akaishi mountains there are more than 500 mature trees that can produce cones. This estimation supported inclusion of this species as one “vulnerable to extinction” on the red list by the Environmental Agency of Japan (2000). However, population size and density are not sufficient for regeneration in habitats where mature trees stand alone, and it is

feared that small populations will disappear in the future.

With regard to genetic variation within the 3 populations (Fuki-sawa, Ohdaira and Tenshi-iwa) of *Picea koyamae*, shown in Table 4, estimated values of N_e (1.23-1.25) and H_e (0.185-0.196) in this study were not clearly different from the summarized values ($N_e=1.32$, $H_e=0.173$) of 195 long-lived tree species (Hamrick & Godt 1992). In comparison with Japanese conifer species, the genetic variation of *P. koyamae* is nearly the same as those of *Cryptomeria japonica* ($N_e=1.33$, $H_e=0.189$; Tomaru *et al.* 1994), *Chamaecyparis obtusa* ($N_e=1.33$, $H_e=0.193$; Uchida *et al.* 1997), and *Pinus pumila* ($N_e=1.38$, $H_e=0.225$; Tani *et al.* 1996), and greater than that of *Abies mariesii* ($N_e=1.08$, $H_e=0.054$; Suyama *et al.* 1997). On the other hand, variation of the Karamatsu-sawa population ($N_e=1.16$, $H_e=0.136$) was rather lower than those of the other populations investigated in this study. In this population, diameter distribution of mature trees shows one peak, and the forest age was estimated to be about 100 years old. An investigation conducted in 1911 showed no old growth at that time (Shirasawa & Koyama 1913). From those evidences, this population might have regenerated at once, about 100 years ago. It is conceivable that the low variation within this population would have been caused by a bottleneck effect through a limited number of mother trees.

With regard to genetic differentiation among the 4 populations of *Picea koyamae* shown in Table 6, the estimated G_{ST} value (0.067) in this study was slightly lower than the mean estimated value (0.084) summarized for 195 long-lived tree species (Hamrick & Godt 1992). G_{ST} values of Japanese conifers were estimated to be 0.034 for *Cryptomeria japonica* (Tomaru *et al.* 1994), 0.030 for *Chamaecyparis obtusa* (Uchida *et al.* 1997), 0.170 for *Pinus pumila* (Tani *et al.* 1996), and 0.144 for *Abies mariesii* (Suyama *et al.* 1997). The former two species have wide (over all of Japan except

TABLE 3. Allele frequencies of 10 allozyme loci in 4 populations.

Locus – Allele	Yatsugatake		Akaishi	
	Fuki-sawa (31 ^{§§§})	Karamatsu-sawa (25)	Ohdaira (32)	Tenshi-iwa (31)
<i>Dia-1</i> [§] <i>a</i> ^{§§}	0.97	1.00	0.97	0.97
<i>b</i>	0.03	0.00	0.03	0.03
<i>Dia-2</i> <i>a</i>	0.60	0.08	0.30	0.47
<i>b</i>	0.40	0.92	0.70	0.53
<i>Fm</i> <i>a</i>	1.00	1.00	1.00	1.00
<i>G2d</i> <i>a</i>	0.42	0.38	0.36	0.45
<i>b</i>	0.58	0.62	0.64	0.55
<i>Got-1</i> <i>a</i>	1.00	1.00	1.00	1.00
<i>Got-2</i> <i>a</i>	1.00	1.00	1.00	1.00
<i>Pgm</i> <i>a</i>	0.48	0.70	0.69	0.68
<i>b</i>	0.52	0.30	0.31	0.32
<i>Shdh</i> <i>a</i>	0.27	0.18	0.34	0.32
<i>b</i>	0.73	0.82	0.66	0.68
<i>Ugpp</i> <i>a</i>	1.00	1.00	1.00	1.00

[§] The locus with -1 in its designation represents a form moving electrophoretically faster than that with -2.

^{§§} Allele *a* stands for the allele moving faster than allele *b* in electrophoresis.

^{§§§} Sample number

TABLE 4. Genetic variation within 4 populations.

Mountains	Population	P(%)	Na	Ne	Ho	He(SE)
Yatsugatake	Fuki-sawa	40	1.50	1.25	0.213	0.196(0.076)
	Karamatsu-sawa	40	1.40	1.16	0.132	0.136(0.062)
Akaishi	Ohdaira	40	1.50	1.23	0.150	0.185(0.072)
	Tenshi-iwa	40	1.50	1.24	0.184	0.196(0.076)

P, percentage of polymorphic loci at a 95% criterion; Na, number of alleles per locus; Ne, effective number of alleles per locus; Ho, average observed heterozygosity; He, average expected heterozygosity.

TABLE 5. Fixation indices (F_{IS}) within 4 populations for polymorphic loci.

Mountains	Population	<i>Dia-2</i>	<i>G2d</i>	<i>Pgm</i>	<i>Shdh</i>
Yatsugatake	Fuki-sawa	-0.121	-0.173	0.110	-0.196
	Karamatsu-sawa	-0.065	0.085	0.160	-0.195
Akaishi	Ohdaira	0.042	-0.136	0.570**	0.318
	Tenshi-iwa	0.044	0.103	0.129	-0.017

** P<0.01.

TABLE 6. Levels of genetic differentiation among 4 populations based on 4 polymorphic loci and on all loci including those that were polymorphic and monomorphic.

Locus	H _T	H _S	G _{ST}
<i>Dia-2</i>	0.450	0.353	0.216
<i>G2d</i>	0.481	0.478	0.005
<i>Pgm</i>	0.462	0.447	0.034
<i>Shdh</i>	0.403	0.395	0.020
Mean [§]	0.449	0.418	0.069
All	0.184	0.172	0.067

[§] Mean on 4 polymorphic loci.

Hokkaido) and continuous distribution areas, the latter two species' distribution areas are restricted to central and northern Japan and are discontinuous. The value of 0.067 in *P. koyamae* is larger than those of the first two species and smaller than those of the latter two species. The G_{ST} value of *P. koyamae* might have been affected by its wide-area con-

tinuous distribution on Honshu Island in the Last Glacial Age.

The dendrogram (Fig. 3) based on standard genetic distances suggests that Ohdaira and Tenshi-iwa populations in Akaishi mountains are very closely related, and that the Karamatsu-sawa population is remote from the other three populations. The two populations in Akaishi mountains might be continuous through some unknown populations in areas which our field survey did not cover. The genetic remoteness of Karamatsu-sawa from the other populations could be explained by the probable bottleneck effect.

The Karamatsu-sawa population is preserved as a forest reserve, but its genetic variation is low and cannot represent that of the species *Picea koyamae*. Therefore, for the purpose of preserving the genetic variation across the species, not only

TABLE 7. Standard genetic distances for all pairs from each of 4 populations.

	Fuki-sawa	Karamatsu-sawa	Ohdaira
Karamatsu-sawa	0.045 (0.042)		
Ohdaira	0.014 (0.012)	0.010 (0.009)	
Tenshi-iwa	0.003 (0.004)	0.024 (0.025)	0.001 (0.003)

The figures in parentheses stand for standard errors of genetic distances.

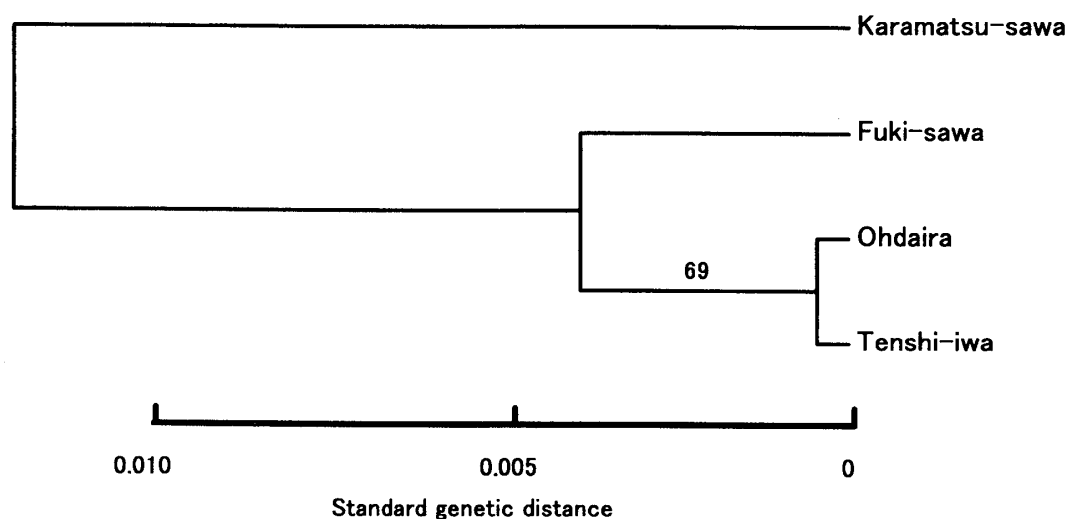


FIG. 3. Dendrogram from UPGMA analysis based on pairwise standard genetic distances. The figure in the phylogenetic tree presents bootstrap probability based on 1,000 replicates.

Karamatsu-sawa but also other populations such as Fuki-sawa or Tenshi-iwa are important. It is desirable that care will be taken to preserve the populations in these other habitats, too. Fortunately, the Ohdaira population is scheduled to be registered as a preserved forest by Yamanashi Prefecture.

“*Picea shirasawae*” was included as *P. koyamae* in this study. There was no clear genetic evidence to suggest the existence of two independent taxa in our results, as follows. If *P. koyamae* and “*P. shirasawae*” are independent taxa and had not mated with each other in any of our samples, the F_{IS} calculated on the assumption of a single random mating population should have a positive value according to “Wahlund’s Principle” (Crow & Kimura 1970). However, table 5 shows that there was no clear result of significantly positive F_{IS} , especially in the Fuki-sawa and Karamatsu-sawa populations which were reported as mixed stands of *P. koyamae* and “*P. shirasawae*” by Shirasawa & Koyama (1913) and Hayashi (1960a). According to the National Forest Agency, 40 individuals of *P. koyamae* and 31 individuals of “*P. shirasawae*” were registered from Karamatsu-sawa population in 1965. This evidence would suggest that “*P. shirasawae*” is not an independent taxon, but rather, represents a morphological variation within *P. koyamae*.

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References

- Constantine, C. C., R. P. Hobbs & A. J. Lymbery. 1994. FORTRAN programs for analyzing population structure from multilocus genotypic data. *J. Hered.* 85:336-337.
- Crow, J. F. & M. Kimura. 1970. *An Introduction to Population Genetics Theory*. Harper & Row, New York.
- Environmental Agency of Japan 2000. *Threatened Wildlife of Japan. Red Data Book 2nd ed. Vol. 8. Vascular Plants*. Japan Wildlife Research Center, Tokyo. (in Japanese).
- Farjon, A. 1990. *Pinaceae*. Koeltz Scientific Books, Königstein.
- & C. N. Page (eds.) 1999. *Conifers, Status Survey and Conservation Action Plan*. IUCN, Cambridge.
- Fu, S., W. Wang, W. Cheng & L. Fu. 1978. *Picea*. In: Chen, W. & L. Fu (eds.) *Fl. Reipub. Popul. Sinicae*. 7: 123-167. (in Chinese).
- Hamrick, J. L. & M. J. W. Godt. 1992. Allozyme diversity in plant species. In: Brown, A. H. D., M. T. Clegg, A. L. Kahler & B. S. Weir (eds.) *Plant Population Genetics, Breeding and Genetic Resources*. pp. 43-63. Sinauer, Sunderland, MA.
- Harris, H. & D. A. Hopkinson. 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. North-Holland Publishing Company, Amsterdam.
- Hayashi, Y. 1960a. *Taxonomical and Phytogeographical Study of Japanese Conifers*. Norin Shuppan, Tokyo. (in Japanese).
- 1960b. Note on Japanese trees and shrubs (4). *Bull. Gov. For. Exp. Stn.* 125: 71-74.
- Katsuki, T. & K. Seido. 1999. Populations of *Picea koyamae* and *P. maximowiczii* in Hakushu-sho, Yamanashi Prefecture. *Trans. Kanto Jpn. For. Soc.* 50: 69-70. (in Japanese).
- Kimura, M. & J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49:725-738.
- Kobayashi, K., J. Yoshikawa, & M. Suzuki. 2000. DNA identification of *Picea* species of the Last Glacial Age in northern Japan. *Jpn. J. Histor. Bot.* 8:67-80.
- LePage, B. A. 2001. New species of *Picea* A. Dietrich (Pinaceae) From the middle Eocene of Axel Heiberg Island, Arctic Canada. *Bot. J. Linn. Soc.* 135:137-167.
- Li, C. C. & D. G. Horvitz. 1953. Some methods of estimating the inbreeding coefficient. *A. J. Hum. Genet.* 5:107-117.
- Minaki, M. 1987. Fossil plants of the Last Glacial Age and its evolutionary significance. *Iden* 41(12):30-35 (in Japanese).
- Nei, M. 1972. Genetic distance between populations.

- Am. Nat. 106:283-292.
- _____. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.
- _____. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.
- _____ & R. K. Chesser. 1983. Estimation of fixation indices and gene diversities. Ann. Hum. Genet. 47:253-259.
- _____ & A. K. Roychoudhury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76:379-390.
- Note, H., S. Okitsu & A. Momohara. 1999. Habitat of *Picea koyamai* Shirasawa and *Picea maximowiczii* Regel ex Mast. in Japan. J. Jpn. For. Sci. 81:236-244. (in Japanese).
- Ota, T. 1993. DISPAN: Genetic distance and phylogenetic analysis. The Pennsylvania State University.
- Shimizu, T. 1989. New names of some Japanese plants. J. Phytogeogr. Taxon. 37: 120.
- _____. 1992. Taxonomy and phytogeography of conifers, with special reference to some subalpine genera (2) The genus *Picea*. Jpn. J. Histor. Bot. 9: 3-11. (in Japanese).
- Shirasawa, Y. & M. Koyama. 1913. Some new species of *Picea* and *Abies* in Japan. Bot. Mag. Tokyo 27: 127-131, pl. 2. (in Japanese).
- Sneath, P. H. A. & R. R. Sokal. 1973. Numerical Taxonomy. W. H. Freeman, San Francisco.
- Suyama, Y., Y. Tsumura & K. Ohba. 1997. A cline of allozyme variation in *Abies mariesii*. J. Plant Res. 110:219-226.
- Suzuki, M. 1991. *Picea* cone-fossils from Pleistocene strata of Northeast Japan. Saito-Ho-on Kai Museum Research Bulletin 59:1-41.
- Tani, N., N. Tomaru, M. Araki & K. Ohba. 1996. Genetic diversity and differentiation in populations of Japanese stone pine (*Pinus pumila*) in Japan. Can. J. For. Res. 26:1454-1462.
- Tomaru, N., Y. Tsumura & K. Ohba. 1994. Genetic variation and population differentiation in natural populations of *Cryptomeria japonica*. Plant Sp. Biol. 9:191-199.
- Tsumura, Y. & K. Ohba. 1993. Genetic structure of geographical marginal populations of *Cryptomeria japonica*. Can. J. For. Res. 23:859-863.
- _____, N. Tomaru, Y. Suyama, M. Na'iem & K. Ohba. 1990. Laboratory manual of isozyme analysis. Bull. Tsukuba Univ. Forests 6:63-95. (in Japanese).
- Uchida, K., N. Tomaru, C. Tomaru, C. Yamamoto & K. Ohba. 1997. Allozyme variation in natural population of hinoki, *Chamaecyparis obtusa* (Sieb. et Zucc.) Endl., and its comparison with the plus-tree selected from artificial stands. Breed. Sci. 47:7-14.
- Wilson, E. H. 1916. The Conifers and Taxads of Japan. The University Press, Cambridge.
- Wright, J. W. 1955. Species crossability in spruce in relation to distribution and taxonomy. Forest Sci. 1:319-349.
- Wright, S. 1951. The genetical structure of population. Ann. Eugen. 15:323-354.
- Yamazaki, T. 1965. Materials for the distribution of vascular plants in Japan (41). Journ. Jpn. Bot. 40:328. (in Japanese).
- _____. 1995. Pinaceae. In: Iwattsuki, K., T. Yamazaki, D. E. Boufford & H. Ohba. (eds.) Flora of Japan, vol. 1 Pteridophyta and Gymnospermae. pp. 266-277. Kodansha, Tokyo.

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